[DOCKET NO. 84N-0154]

DRAFT OF POINTS TO CONSIDER IN THE CHARACTERIZATION OF CELL LINES USED TO PRODUCE BIOLOGICALS (1993)

Submit written comments on this draft to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
rm. 1-23, 12420 Parklawn Dr.
Rockville, MD 20857

Submit written requests for single copies of this draft to:

Congressional and Consumer Affairs Branch (HFM-12)
Food and Drug Administration
1401 Rockville Pike, Suite 200N
Rockville, MD 20852-1448

For further information about this draft, contact:

Center for Biologics Evaluation and Research (HFM-20)
Food and Drug Administration
1401 Rockville Pike, Suite 200N
Rockville, MD 20852-1448
301-402-3190

Comments and requests should be identified with the docket number found in brackets in the heading of this document.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

DATE: JUL 12 1993

FROM: Director, Center for Biologics Evaluation and Research

SUBJECT: Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)

TO: Manufacturers Utilizing Cell Lines for the Production of Biologics

This Points to Consider Document on the Characterization of Cell Lines Used to Produce Biologicals (revised May 1993), is intended to replace the document of the same title issued in 1987. These "Points" are neither regulations nor guidelines, but serve to represent the current consensus of the Center for Biologics Evaluation and Research (CBER) staff.

We intend to continually revise and update this document as necessary in order to improve it's usefulness. You are requested to review and comment on this document. All comments should be addressed to:

Dockets Management Branch
Food and Drug Administration
12420 Parklawn Drive, Room 1-23
Rockville, MD 20857

Kathryn C. Zoon

Kathryn C. Zoon, Ph.D.

Note: Comments should be identified by the docket number 84N-0154.
Two copies of any comments should be submitted.
S. Salter, HFM-630 8/11/93

POINTS TO CONSIDER IN THE CHARACTERIZATION OF CELL LINES USED TO PRODUCE BIOLOGICALS (1993)

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION

Table of Contents

I. INTRODUCTION

This document is concerned with the characterization of cell lines used to produce biological products which are subject to licensure under the U.S. Public Health Service Act and also with the identification of possible adventitious infectious agents from the cell lines which might contaminate the final product. The existing general regulations in 21 CFR 200 et seq. and 21 CFR 600 et seq. and especially 21 CFR 610.18 et seq. embody requirements with objectives that the final product be uniform, consistent from lot-to-lot and free from adventitious infectious agents. This document provides information that may be useful to manufacturers in achieving these objectives, but does not create new requirements or rights.

Advances in biotechnology are occurring rapidly. Each new product should be evaluated in light of its own particular characteristics and the cell line and manufacturing process being used. Therefore, information in this document is subject to change as new and significant findings become available. Accordingly, this discussion should be interpreted as raising scientific issues that manufacturers who produce biological products from cell lines should consider both during product development under investigational new drug applications (INDs) and before submitting product license applications (PLAs). Existing general regulations, 21 CFR 200 series and 600 series, are also broadly relevant and should be consulted.

These points are not all-inclusive. Alternative approaches may well be suitable in specific situations, and certain aspects may not be applicable to all situations. Furthermore the scientific basis for determining the appropriateness of the points specified for consideration here is developing rapidly and more appropriate approaches may be developed in the future. Therefore, the Center for Biologics Evaluation and Research (CBER) will review the adequacy of testing of any cell line on a case-by-case basis.

This document supersedes the "Points to Consider (PTC) in the Characterization of Cell Lines Used to Produce Biologicals (1987)" and reflects a number of changes emanating from several international workshops held since that time (1,2). As stated in the 1987 PTC, the current approach to working with cell lines to produce biological products focuses on:

1. production, identification and characterization of the cell substrate;
2. validation of the manufacturing process for removal and/or inactivation of adventitious agents; and
3. testing of the bulk and final product to assure safety.

However, it should be noted that a number of tests previously recommended have been revised or eliminated. Specifically:

1. Karyology

In 1978 an ad hoc committee met to revise the recommendations on karyology control. The Committee's report was published in 1979 (3). The detailed characterization and monitoring procedures described in 1979 applied specifically and are still applied to diploid cell lines used for the production of, for example, live virus vaccines. However, the utility of karyology for the characterization of continuous cell lines is probably minimal; therefore, routine karyology is not recommended in these circumstances.

2. Tumorigenicity testing

Experience has shown that virtually all continuous cell lines of rodent origin are tumorigenic; therefore, rodent cells need not be tested for tumorigenicity. Human epithelial cells and all cells used for live virus vaccine production should, however, be tested for tumorigenicity. In addition, some special cases regarding somatic cell or gene therapy may require tumorigenicity testing.

3. Oncogene testing

Recent studies indicating that oncogenes may be involved in normal cell growth suggest that testing for endogenous oncogenes is not necessary.

The results of tests described in this document may be submitted to support the acceptability of a cell line to produce a biological product. In general, testing should be performed in compliance with Good Laboratory

Practice requirements (21 CFR 58). These tests should not be interpreted as checklists. Rather, the selection of tests depends on many variables such as the nature of the cell line, the manufacturing situation and the product indication. In addition, the amount of testing that is needed may be greater to support approval of a PLA than that for an IND application. The testing required for initiating clinical trials depends on the product and its use. The points discussed here do not generally address the basis for test selection due to the variety of issues each manufacturer must consider when making these decisions.

The characterization of a cell line intended for use in the manufacture of biologicals includes:

1. history and general characteristics of the cell line;
2. the cell bank system; and
3. quality control testing.

In addition, in many cases there is need for validation studies of virus removal and inactivation by the manufacturing process.

Additional information concerning the testing of cell lines used to produce monoclonal antibodies and recombinant DNA technology products for *in vivo* and select *in vitro* use may be found in the "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (June 1987)" (now under revision), "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (April 1985)" and the supplement to the recombinant DNA

Points to Consider, "Nucleic Acid Characterization and Genetic Stability (1992)." If a cell line or cells are to be returned into humans to produce its biological product(s) *in vivo*, then the "Points to Consider in Human Somatic Cell Therapy and Gene Therapy (1991)" and also the Points to Consider in the Collection, Processing and Testing of Ex-Vivo-Activated Mononuclear Leukocytes for Administration to Humans (1989) should be consulted.

II. HISTORY AND GENERAL CHARACTERISTICS OF THE CELL LINE

A. History of the Cell Line

The history of any cell line used for the production of biological products should include, when possible:

1. age, sex and species of the donor;
2. for human cell lines, the donor's medical history and if available, the results of tests performed on the donor for the detection of adventitious agents;
3. culture history of the cell line including methods used for the isolation of the tissues from which the line was derived, passage history, media used and history of passage in animals, etc.;
4. previous identity testing and the results of all available adventitious agent testing.

B. General Characteristics of the Cell Line

The growth pattern and morphological appearance of the cell line should be determined and should be stable from the master cell bank to the end-of-production cells [Points to Consider: "Nucleic Acid Characterization and Genetic Stability (1992)"]. If there are specific markers that may be useful in characterizing the cell line (such as marker chromosomes, specific surface markers), these should be characterized for stability. If the cells have an identified finite life expectancy, the total number of population doubling levels through senescence should be determined.

III. THE CELL BANK SYSTEM

A. Generation of Cell Banks

Once a cell line is chosen as the biological source of a product, a cell bank system should be generated to assure that an adequate supply of equivalent cells exist for use over the entire life span of the product. In addition to providing a constant supply of starting material, the advantages of a cell bank system include allowing for a detailed characterization of the cell line and decreasing the likelihood and increasing the detection of both cell line cross-contamination and adventitious agent contamination. Ordinarily, the cell bank system would consist of two tiers: a master cell bank (MCB) and a manufacturer's working cell bank (MWCB).

The Master Cell Bank is defined as a collection of cells of uniform composition derived from a single tissue or cell. It is cryopreserved in aliquots stored in the liquid or vapor phase of liquid nitrogen. The MCB for a diploid cell line should be prepared from cells at a low population doubling level.

The Manufacturer's Working Cell Bank (MWCB) is derived from one or more ampules of the MCB. The MCB source cells are expanded by serial subculture up to a passage number selected by the manufacturer and approved by CBER. At that point the cells are combined into one pool, dispensed into individual ampules and cryopreserved to form the MWCB. One or more such ampules from the MWCB would be used for the production of a lot of a biological product. If cells from more than one MWCB ampule are used, the cell suspensions should be pooled at the time of thawing. The population doubling level of cells used for production should not exceed an upper limit based on written criteria established by the manufacturer.

B. Storage of the Cell Banks

Both the MCB and the MWCB should be stored in either the liquid or vapor phase of liquid nitrogen. The location, identity and inventory of individual ampoules of cells should be thoroughly documented. It is recommended that the MCB and MWCB should each be stored in two or more widely separate areas within the production facility as well as at a distant site in order to avoid loss of the cell line.

C. Cell Bank Qualification

1. The Master Cell Bank

Testing to qualify cell banks should be done either on an aliquot of the cell bank or on cell cultures derived from the cell bank, as appropriate. Testing to qualify the MCB includes testing to demonstrate freedom from adventitious agents and identity testing. The testing for adventitious agents should include tests for bacteria, fungi, mycoplasmas and for viruses. Testing for adventitious viruses should include routine *in vivo* and cell culture inoculation tests and any other specific tests that are warranted, based on the passage history of the cell line, to detect possible contaminating viruses. Some of the tests which are relevant in selected circumstances are described in part V. Finally, testing should be performed in most circumstances to determine if the cells produce retroviruses or retrovirus particles. This testing is also described in part V.

Extensive identity testing of the MCB should be done once and should include all tests needed to establish all significant properties of the cells and the stability of these properties throughout the manufacturing process. Such characteristics should include:

a. morphology, as determined by light and electron microscopy;

b. species of origin (and sex, if human);

c. split ratio;

d. data demonstrating that the cells can be used for their intended purpose. If the cells contain an expression system to produce a recombinant DNA-derived protein, data should be obtained to demonstrate the copy number and physical state of the expression system and the quality and quantity of the protein it produces (see Points to Consider on rDNA products);

e. a meaningful test should be performed which will also be performed for routine identity testing of production cultures used for each lot of product; and

f. other such tests which may be useful for demonstrating that the cell bank is comprised of cells with the intended characteristics.

2. Manufacturers' Working Cell Bank

The MWCB being derived from the MCB and propagated for an approved number of passages in tissue culture, only needs to be spot checked for contaminants that may have been introduced from the culture medium. Recommended tests include sterility, mycoplasma, routine virus (in vitro and in vivo) tests and cell line authenticity to check for cell line cross-contamination.

When a manufacturer moves from a serum containing to a serum free defined growth medium,it is suggested that the cells which are weaned into the serum free medium should be recloned to establish a new MCB and MWCB of cells for optimal growth in the defined medium.

IV. PRODUCTION CULTURES AND PRODUCT TESTING

Quality control of cell substrates used for production is an important part of product quality control. Specific areas to be addressed include cell culture media, management of cell cultures, and specific testing.

A. Cell Culture Media

Accurate records should be kept of the composition and source of the cell culture medium. In cases where the manufacturer of a biological product uses a proprietary medium or medium supplement, the manufacturer of the medium or medium supplement may be

required to supply the necessary data directly to CBER, in the form, for example, of a Master File Application.

If serum or additives derived from animal sources are added to the cell culture medium, they should be certified to be free from contaminants and adventitious agents, such as the agent responsible for the production of Bovine Spongiform Encephalopathy. Information should be provided with regard to the identity and source of, and testing for adventitious agents carried out on these additives. Acceptance of certified raw materials based on certification provided by the supplier should be based on a determination by the manufacturer accepting the product that the process used for certification is sufficient.

Since animal serum may produce allergic responses in human subjects, attempts should be made to reduce serum levels required for the propagation of production cell cultures as much as possible. The residual amount of serum or additives in the final product should be determined and shall not exceed 1:1,000,000(21 CFR 610.15(b)).

If porcine trypsin is used in passaging cells, it should be free from adventitious agents, including porcine parvovirus (9 CFR 113.51 and 113.53). Pursuant to 21 CFR 207.31, manufacturers of biological products are requested to provide information regarding the source(s) and control of any bovine- or ovine-derived material(s) (see attachment #1).

Penicillin or other beta lactam antibiotics should not be present in production cell cultures. Minimal concentrations of other antibiotics or inducing agents may be acceptable [21 CFR 610.15(c)]. However, the presence of any antibiotic or inducing agent in the product is discouraged.

B. Management of Cell Cultures

Lot-to-lot characterization of the product and routine monitoring for adventitious agents is part of the quality control of the biological product. It includes testing of production cell cultures and unprocessed and processed cell culture fluids.

Appropriate approaches to quality control of cell substrate depend on the nature of the propagation system used. Cell substrates are propagated as monolayer cultures, in suspension cultures, or in bioreactors, and may be held on a short term, long term, or even on a potentially indefinite basis. When short-term cultures are used, the product is obtained either from a single harvest of cell culture fluid or from multiple harvests. In some cases the quality control testing may need to be performed on each harvest before pooling into the bulk lot.

If the product is an infectious virus, it will usually replicate in one or more of the cell cultures used for routine testing for adventitious viruses. Nonreplicating viruses, used as vectors for gene therapy, may be tested in the usual cell culture tests for the presence of adventitious agents. In such cases a proportion of the vessels

containing the cell substrate prepared for production (commonly, about 10% of the vessels) should be held as control cultures. The uninoculated control cell cultures and fluids are tested for adventitious agents. (This should not be confused with the product identity test, which is typically a procedure in which the virus is neutralized and inoculated into a susceptible cell culture.)

When long term cultures are used, multiple harvests may be pooled into bulk lots at intervals. In these cases quality control testing should be performed on each bulk lot, and, if possible, on cells separated from the production harvest pooled into the specific bulk. The management of cell substrates for the purposes of quality control testing should be designed to optimize sensitivity of the testing. Criteria for termination of long-term cultures should be established and followed.

Testing for bacterial and fungal sterility is generally performed on the unprocessed bulk lot, the final bulk lot and the final product. The unprocessed bulk is the pooled harvests of cell culture fluids that constitutes a homogeneous mixture for manufacture into a unique lot of product. It is important that testing for adventitious agents be performed prior to further processing such as filtration, clarification or other procedures, unless such testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas filtered bulk may not). Final bulk product is a concentrated, purified product in a homogeneous suspension prepared for mixing with excipients and filling into final

containers. The final bulk product is subjected to a variety of lot release tests which often include sterility testing if it is intended to be sterile. Final product should be tested for sterility and endotoxin.

Routine testing for mycoplasmas and *in vitro* and *in vivo* testing for adventitious viruses should be performed on every lot using production cells and unprocessed bulk fluids. If a cell line is known to produce a virus that is routinely present in the unprocessed bulk, testing on a lot-to-lot basis to demonstrate its absence from the product after purification may be required, unless the virus is the product, as is the case in viral vectors for gene therapy. Lot-to-lot testing of production cells for cellular identity should be performed.

The presence of nucleic acid from cell lines in biological products has been discussed as a theoretical risk. A World Health Organization consultative group recommended that this theoretical concern was negligible or absent in products that contained less than 100 pg/dose of cellular DNA (4). Lot-to-lot testing for DNA content in biological products produced in cell lines should be performed and lot release limits established that reflect a level of purity that can be achieved reasonably and consistently.

Other tests which should also be performed on every lot include tests that are required on all products (e.g., general safety) or unique tests that reflect the quality of the specific product of concern. In this document the discussion of testing is limited to

those tests which have specific relevance to products produced in cell lines. Manufacturers are responsible for establishing lot release procedures that provide assurance of all significant aspects of product quality.

V. QUALITY CONTROL TESTING

A. Tests for the Presence of Bacteria and Fungi

For required test procedures, see 21 CFR 610.12.

B. Tests for the Presence of Mycoplasma

Tests for the presence of both cultivable and non cultivable mycoplasmas should be performed. Biological products made in insect cell lines should be tested for both mycoplasma and spiroplasma contamination. Current suggested methods for mycoplasma testing are described in attachment #2 of this document. Acceptable tests for spiroplasmas should be discussed with CBER.

C. Tests for the Presence of Viruses

1. Routine Tests for Adventitious Viruses

The cell cultures should be observed at the end of the production period for viral cytopathic effects and tested for hemadsorbing viruses. If multiple harvest pools are prepared at different times, the cultures should be observed and tested at the time of the collection of each pool.

At the time of production of each unprocessed bulk pool, a proportion of the pool should be inoculated into cell cultures, eggs, and mice as follows:

a. An appropriate volume should be inoculated into monolayer cultures of at least three cell types:

(1) monolayer cultures of the same species and tissue type as that used for production;

(2) monolayer cultures of a human diploid cell culture; and

(3) monolayer cultures of a monkey kidney cell culture.

The sample to be tested should be diluted as little as possible. The cell cultures should be observed for at least two weeks. If the production cell culture is known to be capable of supporting the growth of human cytomegalovirus, the human diploid cell cultures should be observed for at least four weeks. The cultures should be tested for hemadsorption at the end of the observation period.

b. Fluids or lysates of the test sample being characterized should be tested for viruses in animals. In most cases, testing in adult and suckling mice and embryonated hen eggs, as described in 21 CFR 630.35, is appropriate. In some cases, testing in guinea pigs, rabbits or monkeys may also be advisable.

2. Selected testing for adventitious viruses

Species-specific viruses present in rodent cell lines may be detected by mouse, rat, and hamster antibody production tests (MAP, RAP, or HAP). *In vivo* testing for lymphocytic choriomeningitis virus (LCM) including challenge for non-lethal strains is recommended. Human cell lines may be screened for human virus pathogens such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and hepatitis B and C (HBV, HCV) using appropriate *in vitro* techniques. Selection of viruses to be screened should take into account the tissue source and medical history of the patient from which the cell line was derived. Retrovirus testing is discussed below.

Use of other cell cultures also may be appropriate for characterization of cell banks depending on the cell type and source of the cell line being characterized (5). Under certain circumstances, specific testing for the presence of other

transforming viruses, such as papilloma-, adeno- and Herpes 6 viruses, may also be indicated.

3. Tests for Retroviruses

Test samples should be examined for the presence of retroviruses utilizing the following techniques:

a. transmission electron microscopy (TEM);

b. reverse transcriptase (RT) assays (performed in the presence of magnesium and manganese) on pellets obtained from fluids by high speed centrifugation (e.g. 125,000 x g for one hour) at 4^0C; and

c. infectivity assays. For murine retroviruses, amplification of low level contaminants may be achieved by co-cultivation of cells with a highly susceptible cell line, e.g. *Mus dunni* cells (6). The latter cells are susceptible to infection by all tested murine leukemia viruses (MuLVs) except Moloney MuLV, in which case another susceptible cell line, e.g. SC-1 (7), should be used. Fluid from the co-cultures should be further passaged on *Mus dunni* cells and subsequently assayed for MuLV.

A variety of other assays may be useful, depending on the circumstances. Some examples of such assays include viable cell immunofluorescence (IFA) on the infected *Mus dunni* cells using a broadly reactive monoclonal antibody (e.g. HY95) for the detection of ecotropic, xenotropic, mink cell focus-forming and amphotropic viruses; feline S+L- assay using PG4 cells (8) for detection of amphotropic viruses; mink S+L- assay for detection of xenotropic viruses (10) and mouse S+L- assay using D56 (9) cells for detection of ecotropic viruses.

It is often possible to increase the sensitivity of tissue culture assays by first inoculating the test material onto cell lines that can support retroviral growth in order to amplify any retrovirus contaminant that may be present at low concentrations. For non-murine retroviruses, test cell lines should be selected for their capacity to support the growth of a broad range of retroviruses, including viruses of human and non-human primate origin (10, 10a).

Murine cell lines or hybrid cell lines containing a murine component should be considered inherently capable of producing infectious mouse retroviruses. For murine cell lines used for monoclonal antibody production, specific retrovirus testing and identification may be abbreviated. However, the manufacturing process should be validated for removal and/or inactivation of retroviruses. For murine-human hybrids, additional concerns arise. The manufacturer should refer to

the "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1987)" and discuss any proposed testing with the agency on a case-by-case basis.

Probe hybridization/PCR amplification and virus-specific monoclonal antibody detection may provide additional information on the presence or absence of specific contaminants.

D. Tumorigenicity Testing

As noted in the introduction, continuous cell lines derived from rodents need not ordinarily be tested for tumorigenicity. Human epithelial lines and all lines used for live virus vaccine production should, however, be tested. In addition, in some special cases, cells to be used in somatic cell or gene therapy may require tumorigenicity testing.

Systems which may be suitable for *in vivo* testing include:

1. nude mice (Nu/Nu) (11);

2. newborn hamsters, mice, or rats immunosuppressed with antithymocyte serum (ATS) or globulin (ATG) (12,13,14);

3. thymectomized and irradiated mice that have been reconstituted with bone marrow from healthy mice.

In all cases, the inoculum should consist of 10^7 reference cells or test cells suspended in a 0.2 ml volume of serum-free medium administered by the subcutaneous or intramuscular route. At least ten animals should be inoculated with test cells which are at or beyond the end-production level and at least ten with reference tumor cells. At least nine out of ten animals injected with reference cells should show progressively growing tumors. In the case of newborn animals treated with antithymocyte preparations, metastases should also be evident in the group injected with reference cells which might include among others, KB, HT-1080, and FL.

In the test systems using newborn hamsters, mice, or rats, the animals should be injected s.c. or i.m. with 0.1 ml volumes of potent ATS or ATG on the day of birth and on days 2, 7, and 14 of life. A potent ATS or ATG is one which suppresses the immune mechanisms of the animals such that the subsequent inoculation of reference tumor cells on the day of birth routinely produces progressively growing tumors and metastases.

In all test systems, the animals shall be observed and palpated at regular and frequent intervals for the formation of nodules at the sites of injection. Any nodules formed should be measured in two dimensions and the data recorded. Animals showing nodules which begin to regress during the period of observation should be sacrificed before the nodules are no longer palpable and processed for histological examination. Animals with

progressively growing nodules should be observed for 1-2 weeks. Among those without nodule formation, half should be observed for 3 weeks and half for 12 weeks before being sacrificed and processed for histological examination. A necropsy should be performed on each animal and will include examination for gross evidence of tumor formation at the site of inoculation and in other organs such as lymph nodes, lungs, brain, spleen, kidneys, and liver. All tumor-like lesions and the site of inoculation are to be examined histologically. In addition, since some cell lines may form metastases without evidence of local tumor growth, any detectable regional lymph nodes and the lungs of all animals should be examined histologically.

In addition to *in vivo* testing, several *in vitro* test systems are useful for the characterization of cell lines. Both colony formation in soft agarose (15) and growth in organ culture (16,17,18) have been shown to be more sensitive assays for tumorigenicity than tumor formation in nude mice (12). These *in vitro* systems are particularly applicable to continuous cell lines some of which are non-tumorigenic in animals at low passage levels. These tests constitute rapid and inexpensive means of demonstrating the stability or progression of abnormal characteristics over the passage history of a candidate cell line. If, in the hands of the manufacturer, these tests are shown to be at least as sensitive as acceptable animal tests, they may in some cases be substituted for the animal tests.

VI. VALIDATION OF VIRAL ELIMINATION

Traditionally, cell lines used as cell substrates for biologics production have been tested to assure the absence of contamination with adventitious viruses, and cell lines free from such contamination were used. As continuous cell lines have been introduced, it has become necessary to qualify for production, cell lines that produce virus-like particles and even infectious viruses. These efforts have resulted in an enhanced understanding of the significance of virus-like particles in cell lines and demonstrated that certain findings, such as the presence of intracisternal type A particles are only of remote theoretical concern. As experience has been gained with monoclonal antibodies produced in cell lines which produce murine retroviruses, evidence has accumulated that such products can be safe and approaches have been developed to minimize both the potential for contamination of the products with retroviruses and the theoretical risk associated with such contamination. In particular, manufacturers have used manufacturing procedures that include steps which cause inactivation and/or removal of viruses from the product and have performed studies to validate the effectiveness of the procedures. When the manufacturing process is known to eliminate significantly more virus than is present in the unprocessed bulk and the purified product is tested for the presence of virus, there is reasonable assurance of freedom from contamination.

Accordingly, when a cell line used for production of a biologic is known or suspected to contain an infectious virus, studies to validate the effectiveness of the manufacturing process in eliminating that virus may assist in qualifying the MCB. Validation studies assist in the quantitation of risk, but do not of themselves prove absence of risk. They are relevant to evaluation of cell lines carrying any type of virus (e.g., Epstein-Barr

virus, papilloma virus) but risk assessment includes consideration of the type of virus and the potential use of the product. Validation studies are not a means of demonstrating that the introduction of an adventitious virus into the cell cultures during manufacture can be acceptable. Validation that the manufacturing process is suitable in this regard is accomplished by demonstration that the process is suitably controlled so that adventitious agents are not introduced. Thus, studies to validate effectiveness of virus removal are only relevant to evaluation of risk associated with cell lines that are known or suspected to carry infectious viruses. Therefore validation may be accomplished by evaluating the ability of the downstream processing steps to specifically remove and/or inactivate virus from the bulk harvest. The product is "spiked" with virus of high titer before testing selected steps in a scaled-down model of the purification scheme.

A. DESIGN

The design of procedures to validate elimination of virus by a purification process should include consideration of the following variables.

1. Selection of appropriate virus or viruses. The virus(es) to be used may be the virus which is known or suspected to contaminate the cell line or it may be a model virus(es) selected because of its similarity to the virus of concern and practical considerations such as availability of material in high titer and ease of assay. The contaminant may be added in a labeled (i.e., radioactive) or non-labeled form. It may be necessary to use more than one virus when,

for example, the use of one virus does not provide an adequate basis for the evaluation of the adequacy of the process.

2. Scaled Down Manufacturing System. If a scaled-down model of the purification scheme is used for this validation process it should accurately reflect the actual manufacturing process. Bed size, flow rate, flow rate to bed size ratio, buffer types, pH, and concentration of protein, salt, and product should all be evaluated and equivalence to full scale manufacturing demonstrated.

3. Analysis of Step-Wise Elimination of Virus. In many cases it is desirable to evaluate the contribution of more than one manufacturing step to virus elimination. Sufficient virus should be present in the material to be tested before each critical step so that an adequate evaluation of the effectiveness of each step is obtained. In some cases simply adding high titer virus to unpurified bulk and testing its concentration between steps will be sufficient. In other cases adding virus to in-process material will also be needed. The virus titer before and after each step being tested should be determined.

4. Determining Physical Removal versus Inactivation. The type of contribution of each of the purification steps should be identified by determining, when feasible, what portion of the reduction is due to virus inactivation and what portion is due to physical removal of the virus from the product.

5. Kinetics of Inactivation. In some cases the kinetics of virus inactivation at the critical inactivation step should be determined. This type of data is particularly important where the virus is known to be a human pathogen and a completely effective inactivation process is being designed.

6. Estimation of Combined Effects. The combined effects of each individually tested step, on the reduction of virus titer, should be calculated in order to establish the total virus inactivation/reduction of the purification procedure.

7. Regeneration of Columns. When chromatographic procedures are depended upon for virus elimination, it is critical that the validation studies should employ columns as actually used during manufacturing. Routine procedures for the regeneration of columns should be such that the design of the validation study is relevant.

8. Specific Precautions.

a. The validation testing is frequently performed outside the manufacturing facility in order to prevent possible virus contamination of the facility.

b. Care should be taken in preparing the high titer virus preparation to avoid aggregation which may enhance physical

removal and decrease inactivation thus distorting the correlation with the actual manufacturing situation.

c. The virus "spike" should be added to the product in a small volume so as not to dilute or change the characteristics of the product.

d. Small differences in, for example, buffers, media, or reagents, can substantially affect virus clearance.

e. Virus inactivation is time dependent. Therefore, the amount of time a spiked product remains in a particular buffer solution or on a particular chromatography column should reflect the conditions of the full scale process.

f. Buffers and product should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus might be necessary. If the biological product itself has an anti-viral activity, the validation study may need to be performed without the product in a "mock" run, though omitting the biological product or substituting a similar protein that does not have anti-viral activity could affect the behavior of the virus in some manufacturing steps.

g. Many purification schemes use the same or similar buffers or columns repetitively. The effects of this approach should be taken into account when analyzing the data. The effectiveness of virus elimination by a particular process may vary with the stage in manufacture at which it is used.

B. INTERPRETATION:

The purpose of a validation study is to show that a process, when done according to SOP's, will reliably give a certain result. For virus contaminants, it is important to show that not only is the virus eliminated, but that there is excess capacity for virus elimination built into the purification process to assure an appropriate level of safety for the final product. The amount of virus eliminated by the manufacturing process is compared to the amount of virus which may be present in ordinary unpurified bulk product.

To carry out this comparison it is important that an estimate of the amount of virus in the ordinary unpurified bulk is made. When possible, this estimate may be done by assays for infectivity. When such assays are not feasible estimates may be made by using transmission electron microscopy to examine a pellet of ultracentrifuged, unpurified material. The entire purification process should be able to eliminate substantially more virus than is thought to be present in the starting material. The excess that is appropriate depends on the virus of concern and the intended use of the product. For example, for products intended for use in immunocompromised individuals, where the relatively small risk of infection may be very significant, a larger excess in clearance capability

may be indicated. The same increase in the clearance capability would apply in the case of products intended for use in a healthy population.

The following potential limitations of studies to validate elimination of virus removal should be addressed when interpreting study results.

1.The model virus may not behave identically to the relevant virus contaminant.

2.The full-scale process may be different from the scaled down process.

3.Unpredicted similarities or redundancies of buffer solutions or procedures may overestimate virus clearance.

4.The summation of the effects of multiple steps, particularly of steps with little effect, may overestimate the true potential for virus elimination.

5.The ability over time of chromatography columns and other devices used in the purification scheme to clear virus after repeated use may vary.

6.Validation studies should be duplicated and the statistical variation within and between studies evaluated.

7.It is recommended that a purification scheme provide at least one virus inactivation step when infectious virus is known to be present routinely in unpurified bulk.

C. STATISTICS

The validation process should include the use of a statistical analysis of the data to evaluate the results. The study design should be statistically valid to support the conclusions reached.

D. REVALIDATION

Whenever significant changes in the production or purification process are made, the effect of that change on virus clearance should be considered and the system revalidated as needed. For example, it is not unusual for changes in production processes to cause significant changes in the amount of virus produced by the cell line or removed by a particular manufacturing step.

VII. CONCLUSION

The FDA's Vaccines and Related Biological Products Advisory Committee meeting of August 21, 1990, reviewed the approach of several manufacturers to remove retrovirus from their products. Proceedings of this meeting emphasized the value of many strategies including:

A. thorough characterization/screening of the cell substrate starting material in order to identify what virus contaminants are present;

B. determination of the human tropism of the contaminants;

C. incorporation of validated virus inactivation and removal steps into the manufacturing process;

D. careful design of the virus validation studies to avoid pitfalls and provide interpretable results; and

E. use of different methods of virus inactivation or removal in the same manufacturing process in order to achieve maximum virus clearance.

Validation studies should be discussed with CBER at the earliest possible time during pre-IND meetings and then again before phase III studies to make sure no outstanding issues remain prior to filing a license application. The successful use of these quality control elements should provide an approach for producing safe biological products.

VIII. REFERENCES

1. Continuous Cell Lines as Substrates for Biologicals. Vol. 70 Developments in Biological Standardization, 1989.

2. Continuous Cell Lines: Current Issues. Developments in Biological Standardization, 1992.

3. Moorehead, P, Earley, EM, Frieling, K, Jacobs, P, Litivin, J, Vetter, R, Petricciani, J, von Seefried, A (1979) J. Biol. Standard., 7, 397-404.

4. Report of a WHO Study Group (1987) Acceptability of cell substrates for production of biologicals. World Health Organization Technical Report Series 747.

5. Jacobs, JP, Magrath, DI, Garrett, AJ, Schild GC (1981) J. Biol. Standard. 9: 331-342.

6. Lander, MR and Chattopadhyay, SK. (1984) J. Virology 52:695-698.

7. Hartley,RH, and Rowe, WP. (1975) Virology 65:128-134.

8. Bassin, RH, Ruscetti, S, Ali, I, Haapala, DK, and Reins, A. (1982) Virology 123:139-151.

9. Bassin, RH, Tuttle, N, and Fischinger, PJ. (1971) Nature 229:564-566.

10. Peebles, PT (1975) Virology, 67. 288-291.

10a Sommerfelt,MA,Weiss,RA, (1990) Virology 176,58-69.

11. Giovanela, BC, et al. (1978) Cancer, 42, 2269-2281.

12. Philips, B and Gazet, JC (1968) Nature, 220, 1140-1141.

13. Wallace, R. Vasington, PJ, Petricciani, J. C (1971) Nature, 230, 454-455.

14. Van Steenis, G, van Wezel, AL (1982) Develop. Biol. Standard., 50, 37-46.

15. Freedman, VH, Shin, S, (1974) Cell, 3, 353-359.

16. Petricciani, JC, Levenbook, IS, Wierenga, ED, Jackson, L, Tiches, K, Locke, R, Koyfman, V, Yateman, T (1983) J. Tissue Culture Methods, 8, 177179.

17. Petricciani, JC, Levenbook, IS, Locke, R (1983) Investigational New Drugs, 1, 297302.

18. Noguchi, PD, Johnson JB, O'Donnell, R, Petricciani, JC (1978) Science, 199, 980-982.

Attachment #1

Letter to the Manufacturers of Biological Products

May 3, 1991

Dear Biologic Product Manufacturer:

The Center for Biologics Evaluation and Research (CBER) is seeking clarification of the procedures and precautions used in controlling materials of bovine or ovine origin used in the manufacture of biologic products intended for administration to humans. This will assist CBER in evaluating the impact of evolving information regarding infectious agents potentially present in materials from bovine or ovine sources (e.g., spongiform encephalopathies).

We are therefore requesting, pursuant to 21 CFR 207.31, that manufacturers of biologic products provide information regarding the source(s) and control of any bovine- or ovine-derived material(s) used in preparing products to be administered to humans for prophylaxis, therapy, or diagnosis. This request is not only for information relating to material that is directly incorporated into the product, but also for information on any materials used in manufacturing (e.g., enzymes, cell culture components, chromatographic media, etc.).

Some specific examples of materials that are, or may be, of bovine or ovine origin include bovine fetal serum, bovine serum albumin, fetuin, proteolytic enzymes (e.g., protease, trypsin, chymotrypsin, etc.), deoxyribonucleases (this is not intended to be a complete listing). If you are unsure of the origin of a component used in the preparation of your products, please obtain this information from the supplier.

Please submit the following information regarding each biologic product that you manufacture under an accepted product license, pending license application or amendment, or investigational new drug application (IND) (This information should not be submitted to your license, license application or amendment, or IND; see instructions below):

The name and status (licensed, license pending, or IND) of each biologic product.

For each product, a list of the material(s) derived from bovine or ovine sources used directly in the product or in manufacturing. If no material from bovine or ovine sources is used, indicate "none" in response.

The name and address of the supplier(s) of each bovine- or ovine-derived material.

A description of the controls utilized by you and the supplier(s) of bovine- or ovine-derived material(s) to assure and document the health and country of origin of the animals used in production of these materials.

A description of the testing performed on each lot of bovine- or ovine-derived material, including the acceptance criteria used. Indicate if the testing is performed by you or the supplier. If performed by the supplier, indicate if you receive detailed test results or summary information.

We request that you submit this information within 60 days of the above date to:

Gerald V. Quinnan, Jr., M.D.
Acting Director
Center for Biologics Evaluation and Research
Division of Biostatistics and Epidemiology
Attention: HFB—250, Building 29—BSE
8800 Rockville Pike
Bethesda, MD 20892

Sincerely yours,

Gerald V. Quinnan, Jr., M.D.
Acting Director
Center for Biologics
Evaluation and Research

Attachment#2

RECOMMENDED PROCEDURES FOR DETECTION OF MYCOPLASMA CONTAMINATION IN BIOLOGICAL PRODUCTS PRODUCED IN CELL SUBSTRATES

Each licensed biological product produced in cell substrates (e.g., viral vaccines, monoclonal antibodies, immunological modulators, interferon and other cytokines, erythropoietin, growth factors, and similar products) must be tested to ensure the absence of mycoplasmal contamination. For most such products, testing should be performed on the virus seed and/or master cell banks, cell substrate and a representative portion (not more than 10 percent) of each working cell stock used for manufacture of the product. Each lot of product harvest concentrate should be tested prior to clarification, filtration, purification, and inactivation, although testing at this stage of the manufacturing process may not be appropriate for all products. Prior to testing, the product harvest concentrate sample should generally be stored between 2 and 8° C for 24 hours or less or at -60° C or lower for 24 hours or more.

As specified in 21 CFR 610.30, mycoplasmal contamination testing must be performed by both the agar and broth media procedure and the indicator cell culture procedure or by a procedure demonstrated to be comparable. The procedural steps recommended for performing both of these procedures are provided below.

The Center for Biologics Evaluation and Research will provide guidance regarding any or all aspects of these procedures.

A. AGAR AND BROTH MEDIA PROCEDURE

(1) Each lot of agar and broth medium should be free of antibiotics except for penicillin, and each lot of medium should be examined for mycoplasmal growth-promoting properties. To demonstrate the capability of the media to detect known mycoplasma contaminants, use the mycoplasmal cultures specified below in (3)(i) as positive controls.

(2)(i) Inoculate no less than 0.2 milliliter (ml) of the product harvest concentrate sample in evenly distributed amounts over the surface of 2 or more agar plates of 1 medium formulation.

(ii) Inoculate no less than 10 ml of the product harvest concentrate sample into a flask containing 50 ml of broth medium which is incubated at 36 + 1° C.

(iii) Test 0.2 ml of the broth culture on the 3rd, 7th, and 14th days of incubation by subculture onto 2 or more agar plates of the same medium formulation as that used above in (i).

(iv) Incubate 2 of the initial isolation plates and 2 each of the three subculture plates in a 5 to 10 percent carbon dioxide in nitrogen and/or hydrogen atmosphere containing less than 0.5 percent oxygen during the test incubation period.

(v) Incubate all culture agar plates for no less than 14 days at 36 + 1° C and observe them microscopically at 10 time magnification (100x) or greater for growth of mycoplasmal colonies.

(3)(i) Include in each test at least 2 known mycoplasma species or strains as positive controls, 1 of which should be a dextrose fermenter (i.e., M. pneumoniae strain FH or equivalent species or strains) and 1 of which should be an arginine hydrolyzer (i.e., M. orale strain CH19299 or equivalent species or strains). Positive control cultures should be not more than 15 passages from isolation and should be used in a standard inoculum of 100 colony forming units (CFU) or 100 color-changing units (CCU) or less.

(ii) Include uninoculated agar medium as a negative control.

(4) Interpret the results of the procedure according to the specification detailed below in (C)(1-4).

B. INDICATOR CELL CULTURE PROCEDURE

(1) Using a Vero cell culture substrate, pretest the procedure by using the mycoplasmal cultures specified below in (3)(i) as positive controls to demonstrate the capability of the cell substrate to detect known fastidious mycoplasmal contaminants. An equivalent indicator cell substrate may be acceptable if data demonstrate at least equal sensitivity for the detection of known mycoplasmal contaminants.

(2)(i) Inoculate no less than 1 ml of the product harvest concentrate samples to 2 or more indicator cell cultures grown on cover slips in dishes or equivalent containers.

(ii) Incubate the cell cultures for 3 to 5 days at 36 + 1° C in a 5 percent carbon dioxide atmosphere. Examine the cell cultures for the presence of mycoplasmas by epifluorescence microscopy using a DNA-binding fluorochrome, such as bisbenzimidazole or an equivalent stain.

(3)(i) Include in each test 2 known mycoplasma species or strains as positive controls (i.e., M. hyorhinis strain DBS 1050, M. orale strain CH19299, or equivalent species and strains), using an inoculum of 100 CFU or 100 CCU or less.

(ii) Include as a negative control a non-infected indicator cell culture.

(4) Interpret the results of the procedure according to the specifications detailed below in (C)(i)-(iv).

C. INTERPRETATION OF RESULTS

(1) For the agar and broth media procedure, compare the appearance of the media inoculated with the product to that of the positive and negative controls.

(2) For the indicator cell culture procedure, using 600 times magnifications (600x) or greater, compare the microscopic appearance of the cultures inoculated with the product to that of the positive and negative cell controls.

(3) Marked cytopathic effects or nuclear chromatin fragmentation caused by virus infection that affect the interpretation of the results can be minimized by using a specific neutralizing viral antiserum or a nonpermissive cell culture substrate. The antisera should also be added to the positive and negative controls.

(4) The product is considered satisfactory for manufacture if both the agar and/or broth media procedure and the indicator cell culture procedure show no evidence of mycoplasmal contamination (i.e., growth) and thus resemble the negative control(s) for each procedure.

(5) If mycoplasmas are recovered, confirmatory testing to establish the species may be useful in determining the probable source of contamination.